## REMARKS

Reconsideration of the above-identified patent application in view of the amendment above and the remarks below is respectfully requested.

Claim 31 has been canceled in this paper. Claim 1 has been amended in this paper. No new claims have been added in this paper. Therefore, claims 1-28 are pending and are under active consideration.

Support for the present amendment to claim 1 may be found in the present specification, for example, in the paragraph spanning pages 7-9.

Claims 1-2 and 4-28 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Eads et al. (Nucleic acids Research 2000 Vol. 28 p. 32) in view of Solinas et al. (Nucleic acids Research 2001 Vol. 29 p. e96)." In support of the rejection, the Patent Office repeats its reasons of record and then states the following:

> The reply traverses the rejection. A summary of the arguments presented in the reply is provided below with a response to arguments following.
>
> The reply asserts that Scorpion primers of Solinas et al. are only used for genomic DNA for the analysis of SNPs or mutations whereas the primers of Eads et al. are used in methylation analysis (p. 19 last paragraph). The reply provides an analysis of the method steps involved in bisulfite treatment (p. 129 last paragraph and p. 20 1st paragraph-3rd paragraph). The reply asserts that due to chemical and physical differences between genomic DNA and bisulfite-treated DNA one would not have used Scorpion primers used in connection to genomic DNA to use with bisulfite-treated DNA. The reply points to several advantages of using Scorpion primers (p. 21 1st paragraph).
>
> The arguments presented in the reply have been fully reviewed but have not been found persuasive.

The reply asserts that the combination of using scorpion primers in a methylation technique is not obvious. It is noted that the argument there are fundamental differences between bisulfite DNA and genomic DNA which has not been pointed out in a particular reference of by a declaration of one skilled in the art and therefore the Attorney's arguments cannot take the place of evidence on the record....However, with regard to the assertion that the skilled artisan would not use Scorpion primers in a bisulfite treated DNA assay has been reviewed but have not been found persuasive. Solinas et al. teaches using scorpion primers in a PCR assay to detect mutations in target samples.

Specifically the reply asserts that the genomic DNA of Solinas et al. differs chemically and physically from the bisulfite-treated DNA of Eads et al. in and that the bisulfite treated DNA is strongly fragmented and therefore has a large amount of false positive signals. Grunau et al. (Nucleic Acids Research 2001 Vol. 29 p. e65) teaches the basic protocol for using bisulfite treated DNA in a PCR assay (p. 3 1st paragraph). Grunau et al. teaches that optimization parameters and incubation times must be determined to determine the DNA degradation during bisulfite treatment, however, PCR products can be generated (abstract). Therefore, Grunau et al. teaches that although bisulfite treated DNA is fragmented PCR amplification can be performed.

Further, the ordinary artisan would be motivated to look for mutations or changes between two targets of bisulfite-treated DNA to make an assertion of a population. There has been no evidence presented that bisulfite treated DNA reacts differently to genomic DNA with regard to the use of Scorpion primers and therefore it would be obvious to use Scorpion primers with any form of DNA including bisulfite treated DNA.

The reply asserts that bisulfite treated DNA contains bases that are not present in DNA, that bisulfite treated DNA consists of large stretches of three bases, that bisulfite treated DNA is single stranded (p. 20 1-3rd paragraphs). However, the art teaches that although genomic and bisulfite DNA has these physical differences, both of these targets can be used in a PCR assay with optimization. As taught by Grunau et al. bisulfite treated DNA requires optimization parameters and incubation times to be determined, however, PCR products can be generated from bisulfite treated DNA.

Applicants respectfully traverse the subject rejection.

As noted in the response to the previous office action, an important advantage of the present invention is the ability to analyze short DNA fragments. This is particularly relevant for the following two reasons: (1) Fragmentation of DNA in sample material; and (2) Additional fragmentation by bisulfite-treatment. Each of the aforementioned reasons is discussed below in greater detail.

Fragmentation of DNA in sample material

An important application of methylation analysis is in the early detection of disease in bodily fluids and in tissue samples, in particular, in paraffin-embedded tissue samples. In this area, methylation analysis has several principal advantages compared to conventional molecular diagnostic methods as RNA is extremely unstable and proteins are not amplifiable. However, one problem in analyzing these sample types is their high degree of DNA fragmentation. Tissue samples are commonly fragmented as a consequence of the fixation methods used. Usually paraffin is applied as a fixation reagent when cutting the samples into slides for further histological analysis. Such fixation, however, also leads to cross-links between the genomic DNA and between DNA and proteins. As a consequence, DNA isolated after paraffin fixation is often fragmented (see U.S. Patent Application Publication No. US2008/0220418). In addition, DNA in bodily fluids (e.g., blood, plasma, urine) often derives from cells which underwent processes like apotosis or aging and, therefore, is fragmented. Furthermore, such DNA is very easily attackable by DNAses (see U.S.S.N. 11/910,887).

Additional fragmentation by bisulfite-treatment

In addition to fragmentation in the sample material, DNA is typically further fragmented as a result of bisulfite treatment. The degree of fragmentation depends on the reaction conditions. Typically, the longer the reaction and the higher the reaction temperature, the higher is the degradation rate. On the other hand, long reaction times are required to achieve a complete DNA-conversion. The reaction product of a bisulfite conversion is a complex mixture of DNAs of different lengths of which a part might be incompletely converted (see Grunau et al.).

The claimed scorpion method is particularly well-suited for the analysis of short DNA fragments and, thus, for the analysis of DNA derived from tissue samples or bodily fluids. This property would have been surprising to a person of ordinary skill in the art as this was neither taught nor suggested by Eads et al. and/or Solinas et al. In particular, a person of ordinary skill in the art, applying Scorpion for mutation analysis, would not have been confronted to the same extent with the problem of fragmented DNA as mutation analysis does not involve a bisulfite conversion.

Applicants are submitting herewith additional experimental data showing that the present invention allows a quantitative determination of the methylation degree of prostate biopsies. (If the Patent Office wishes, Applicants are willing to submit this data in the form of a declaration.) In the experiment, fragments of 67 bp were analyzed. The conventional methods used by the applicant require an amplificate length of at least 150 bp ("QM"-Assay, see U.S. Patent Application Publication No. US2005/0287553).

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 3 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over Eads et al. (Nucleic acids Research 2000 Vol. 28 p. e32) in view of Solinas et al. (Nucleic acids Research 2001

Vol. 29 p. e96) as applied to Claims 1-2 and 4-28 and in further view of Berlin et al. (US Patent Application Publication 2006/0183128 August 17, 2006)." In support of the rejection, the Patent Office repeats its reasons of record and then states the following:

> The reply traverses the rejection. A summary of the arguments presented in the reply is provided below with a response to arguments following.
>
> The reply asserts that the combination of Eads et al. and Solinas et al. is not obvious in view of using Scorpion primers in a bisulfite treated assay (p. 22 last full paragraph).
>
> The arguments presented in the reply have been fully reviewed but have not been found persuasive.
>
> As discussed in the rejection of the claims over Eads et al. and Solinas et al. presented above, the combination of Eads et al and Solinas et al. to make and use the claimed method is obvious in view of the teachings of Solinas et al. which states that Scorpion primers can be used to detect mutations in any sample, and the teachings of Eads et al. which teaches the process of making bisulfite treated DNA.

Applicants respectfully traverse the subject rejection. Claim 3 depends from claim 1. Claim 1 is patentable over Eads et al. in view of Solinas et al. for at least the reasons given above. Berlin et al. fails to cure all of the deficiencies of Eads et al. and Solinas et al. with respect to claim 1. Therefore, based at least on its dependency from claim 1, claim 3 is patentable over the applied combination of Eads et al., Solinas et al. and Berlin et al.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1-28 stand provisionally rejected "on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claim 1-4, 15-16, 18 of copending Application No. 11716207 in view of Solinas et al. (Nucleic acids Research 2001 Vol. 29 p. e96)."

In response to the above, Applicants respectfully request that the subject provisional double patenting rejection be held in abeyance at least until the Patent Office has allowed one of the two patent applications at issue.

Claims 1-2 and 4-28 stand provisionally rejected "on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claim 1-2, 11, 14, 18-19 and 27 of copending Application No. 10482433 in view of Solinas et al. (Nucleic acids Research 2001 Vol. 29 p. e96)."

In response to the above, Applicants respectfully request that the subject provisional double patenting rejection be held in abeyance at least until the Patent Office has allowed one of the two patent applications at issue.

In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is

required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

Kriegsman & Kriegsman

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Dated: March 5, 2009

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on March 5, 2009

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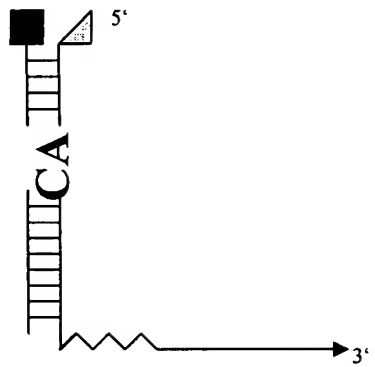
Edward M. Kriegsman

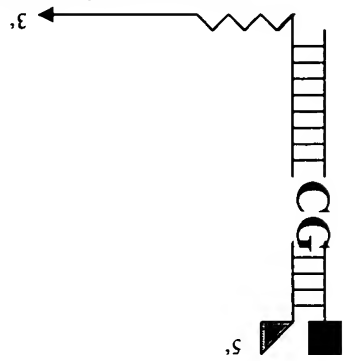
# Quantitative Methylation Detection

# Principle of Quantitative Scorpion Assay

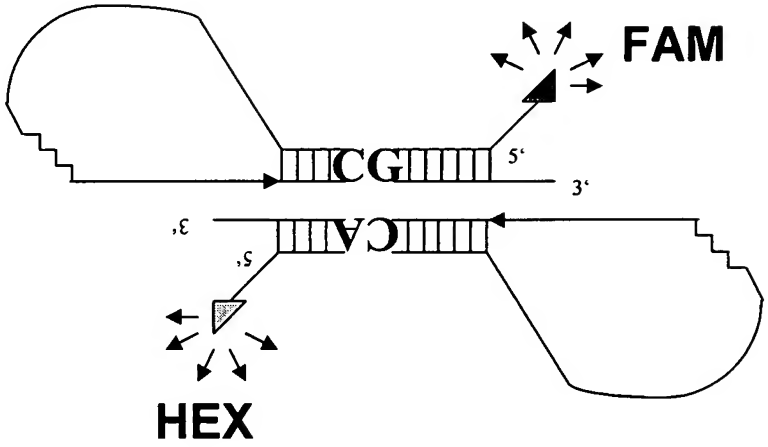



Forward Scorpion Primer

Reverse Scorpion Primer




RealTime PCR




- 1 very short amplicon
- detection of same CpGs with 2 scorpions on complementary strands of the amplicon

- FAM-Scorpion binds to methylated DNA
- HEX-Scorpion binds to unmethylated DNA

# Assay Design for the GSTp1 Gene

LOCUS HSGSTPIG, Human glutathione S-transferase pi gene, ACCESSION X08058

**Location of the MethyLoop Assay in the CpG island of the GSTp1 Gene**

```
1081 gccCGgggtg cagCGgcCGc CGgggctggg gcCGgCGgga gtcCGCGgga ccctccagaa
1141 gagCGgcCGg CGcCGtgact cagcactggg gCGgagCGgg gCGggaccac ccttataagg
1201 ctCGgaggcC GCGaggcctt CGctggagtt tCGcCGcCGc agtcttCGcc accagtgagt
1261 aCGCGCGgcc CGCGtcccCGgggatggggct cagagctccc agcatggggc caaccCGcag
1321 catcaggccC GggctccCGg cagggctcct CGcccacctC GagaccCGgg aCGggggcct
1381 aggggaccca ggaCGtcccc agtgcCGtta gCGgctttca gggggccCGg agCGcctCGg
1441 ggagggatgg gacccCGggg gCGgggaggg ggggcaggct gCGctcacCG CGccttggca
1501 tcctccccCG ggctccagca aacttttctt tgttCGctgc agtgcCGccc tacacCGtgg
```

**Bisulfite Primer:**

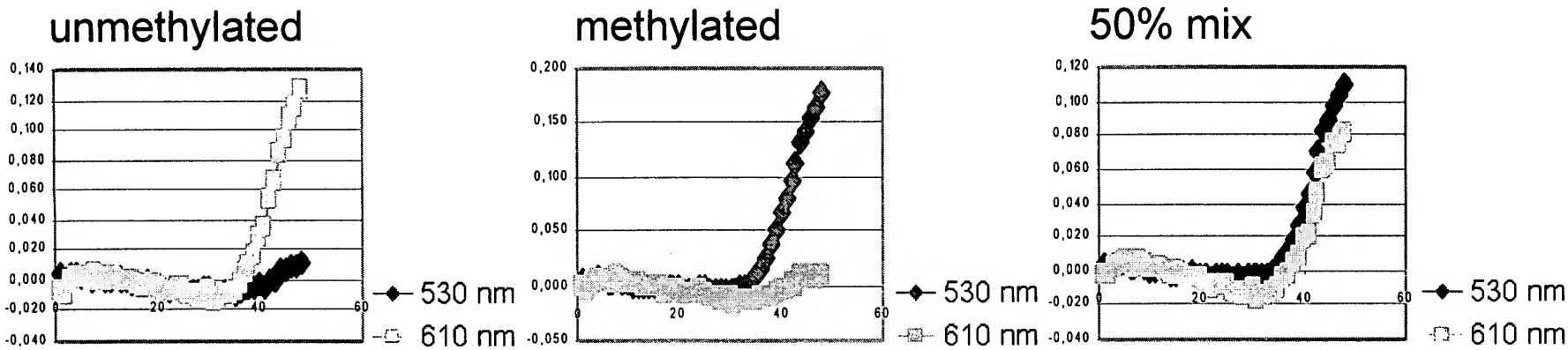
forward GtAGTtTTYGttAttAGTGAGTA (Y=C/T wobble)
reverse TaCTaaaAaCTCTaAaCCCCATC

**Scorpion Primer and Duplex Quencher:**

HEX-aaaACaCaaaCCaCaCa-X-GtAGTtTTYGttAttAGTGAGTA + tGtGGtttGtGTttt-BHQ1 (X=C18 spacer)
FAM-TACGCGCGGTTCG-X-TaCTaaaAaCTCTaAaCCCCATC + AACCGCGCGTA-BHQ1 (X=C18 spacer)

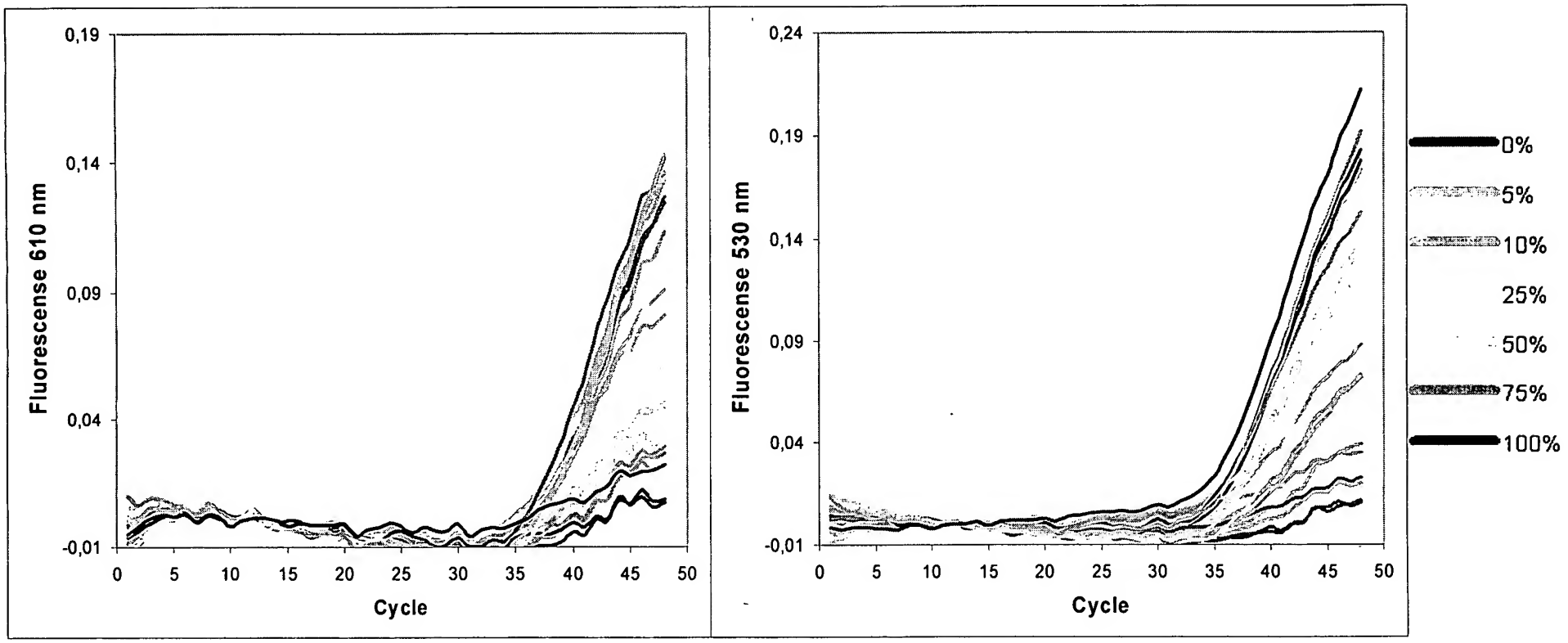
- amplicon lenght: 67 bp
  (Epigenomics standard QM assay in average 150 bp)
- detection of Co-Methylation of 5 CpGs sites

# Signal Ratio Depends on Methylation of Template DNA



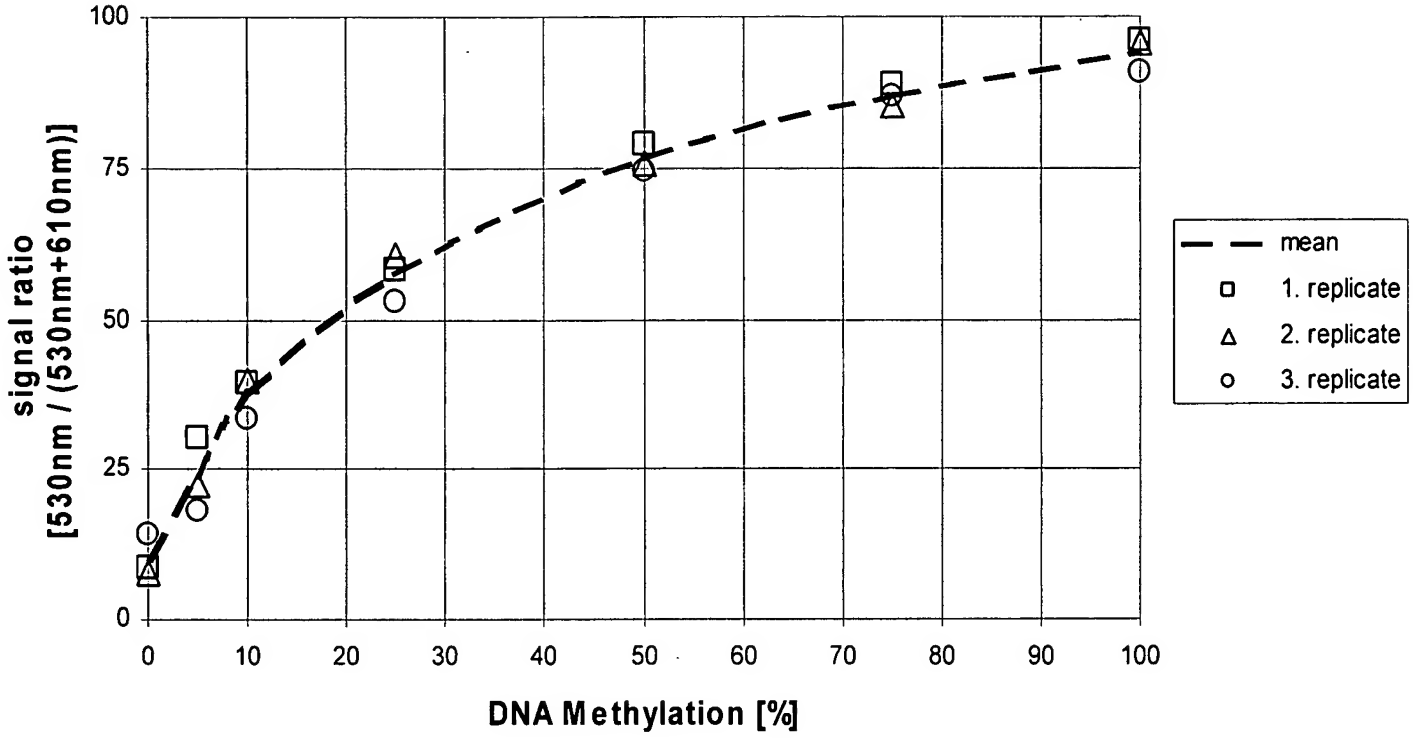

- 2 Scorpions analysing the same CpGs
- methylation specific signals
- parallel detection in different channels

# Calibration Experiment 1 ng



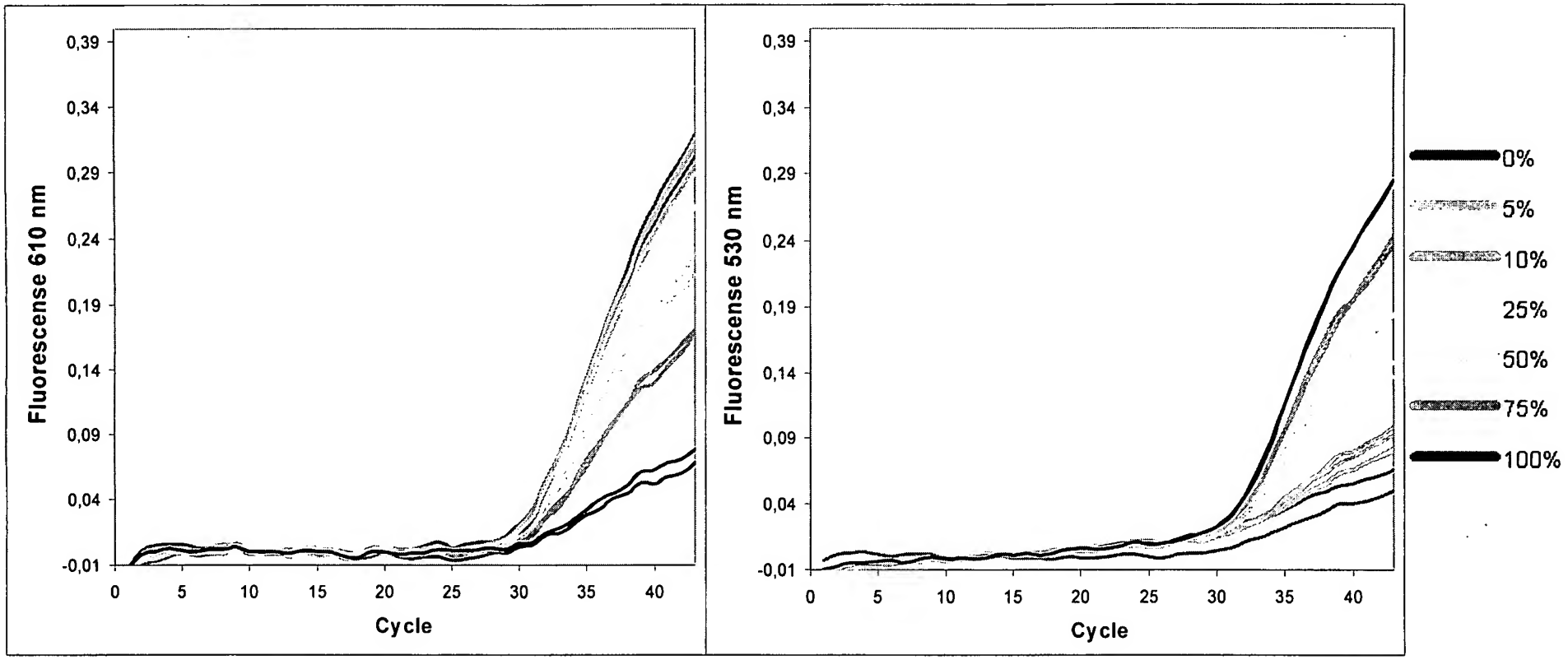

- detection of DNA mixtures with different % methylation
- 1 ng total DNA input
- determination final fluorescense intensities

# Calibration Curve 1 ng



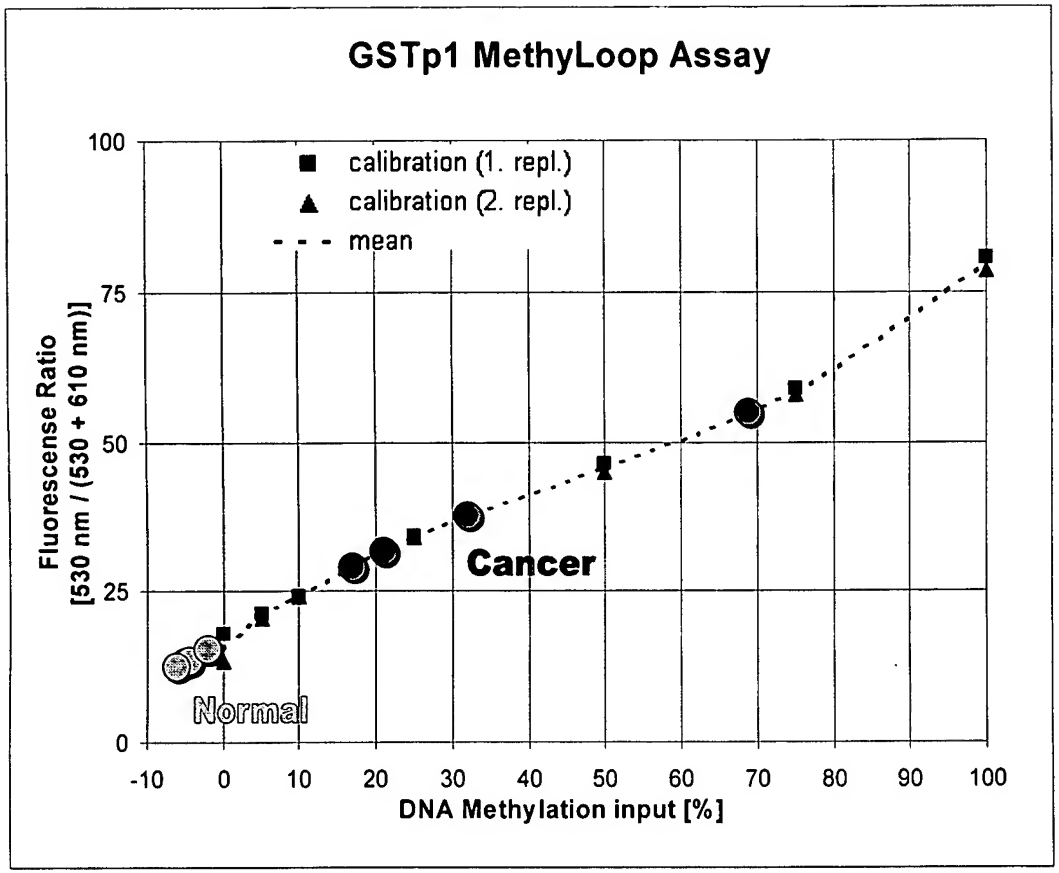

- signal ratio from 3 replicates of different methylation levels
- reproducable quantification of methlyation level
- 5 % methylation detection limit

# Calibration Experiment 20 ng




- detection of DNA mixtures with different % methylation
- 20 ng total DNA input
- determination of final fluorescense intensities

# Prostate Cancer Detection




| Sample | Methylation in GSTp1 [%] |
|---|---|
| Cancer 1 | 32 |
| Cancer 2 | 21 |
| Cancer 3 | 69 |
| Cancer 417 | |
| Normal 1 | 0 |
| Normal 2 | 0 |
| Normal 3 | 0 |
| Normal 4 | 0 |

- 8 prostate needle biopsy
- each sample contains of 10 section (10 µm) from paraffin embedded cores
- processed with Epigenomics' invented DNA bisulfite treatment workflow
- methylation determination by GSTp1 MethyLoop Assay from 20 ng bisDNA

# PCR protocol

| component | final concentration in a 20µl reaction |
|---|---|
| FastStart Master for Hybridisation Probes (Roche Diagnostics) | 1 x |
| MgCl2 | 2,5 mM (final) |
| forward primer | 0,5 µM |
| reverse primer | 0,5 µM |
| forward scorpion - | 0,25 µM |
| reverse scorpion | 0,25 µM |
| quencher for forward scorpion | 0,25 µM |
| quencher for reverse scorpion | 0,25 µM |
| DNA | 10 µl |

| oligonucleotides: | |
|---|---|
| forward primer | GtAGTtTTYGttAttAGTGAGTA Y=C/T wobble or dSpacer |
| reverse primer | TaCTaaaAaCTCTaAaCCCCATC |
| quencher for forward scorpion | FAM-TACGCGCGGTTCG-X-TaCTaaaAaCTCTaAaCCCCATC |
| quencher for reverse scorpion | HEX-aaaACaCaaaCCaCaCa-X-GtAGTtTTYGttAttAGTGAGTA |
| forward scorpion | AACCGCGCGTA-BHQ |
| reverse scorpion | tGtGGtttGtGTttt-BHQ |
| X=C18 spacer, FAM=5'-Fluorescein, HEX=5'-HEX, BHQ1=3'-BlackWholeQuencher | |

| cycling program | | | |
|---|---|---|---|
| initial denaturation at | 95 °C | 10 min | |
| 50 cycles with | 95 °C | 10 sec | (20 °C/s) |
| | 50 °C | 0 sec | (20 °C/s) detection at 530 and 610 nm |
| | 50 °C | 30 sec | (20 °C/s) |
| | 72 °C | 10 sec | (20 °C/s) |